CONCISE COMMUNICATION

Bactericidal activity and synergy studies of BAL9141, a novel pyrrolidinone-3-ylidenemethyl cephem, tested against streptococci, enterococci and methicillin-resistant staphylococci

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BAL9141 has been reported to have inhibitory activity against methicillin-resistant *Staphylococcus aureus* (MRSA), many enterococci, and streptococci with various resistant patterns. BAL9141 potency was assessed by time–kill curves alone or with subinhibitory concentrations of gentamicin (MIC/4). BAL9141 exhibited bactericidal activity alone against all the streptococci and staphylococci. Among ampicillin-susceptible enterococci, BAL9141 was bactericidal against some strains, but no BAL9141 inhibition was observed of ampicillin-resistant *Enterococcus faecium*. The activity of BAL9141 with gentamicin was slightly enhanced (not synergy) or indifferent against staphylococci. BAL9141 demonstrated bactericidal action alone against *Enterococcus faecalis* and some *E. faecium* strains (-4.8 to -6.0 $\log_{10}$ CFU/mL), but static effects were also noted. Drug interactions with gentamicin showed early synergy (4–8 h) for all enterococci, and indifference or synergy at 24 h (no antagonism). BAL9141 ($\leq$8 mg/L) showed promising bactericidal activity alone and synergy with gentamicin against some of the vancomycin-resistant enterococci tested.

Keywords BAL9141, MRSA, bactericidal

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BAL9141 is an antistaphylococcal cephalosporin with additional antimicrobial qualities similar to those of a 'fourth-generation' cephalosporin. BAL9141 showed excellent in vitro activity against multiresistant *Staphylococcus* spp. (including methicillin-resistant strains), streptococci, and a wide range of Gram-negative pathogenic bacteria [1–3]. The anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity of BAL9141 originates from its high affinity for and efficient inhibition of PBP2′. The PBP2′ functions as a transpeptidase, and is not efficiently inhibited by many clinically available β-lactams, such as carbapenems, cephalosporins and penicillins. Since BAL9141 shows all the properties of an advanced-spectrum cephalosporin, in addition to the greater Gram-positive activity, we investigated the bactericidal action and the possibility of synergistic interactions of the β-lactam–aminoglycoside combination.

BAL9141 activity was tested against streptococci, staphylococci and enterococci. The strains were recent clinical isolates from bacteremic patients, standard quality control strains [4,5] or well characterized resistant organisms. The clinical isolates were from different nations and continents (USA, Canada, Europe, and Latin America), forming a diverse geographic sample. Representatives of various resistant phenotypes pertinent to each genus or species group were included (Tables 1 and 2). These isolates were, however, susceptible to BAL9141 by interpretive criteria established for this study [3].

The manufacturer (Basilea Pharmaceuticals, Basel, Switzerland) supplied the BAL9141 powder; and the gentamicin was purchased from Sigma Chemicals (St Louis, MO, USA).

Bactericidal activity as well as synergy was determined by time–kill curve methodology,

Table 1 BAL9141 bactericidal activity tested by time–kill curves against 19 strains of Gram-positive cocci at concentrations of 4, 8 and 16 mg/L

Organism/strain number	Susceptibility (MIC (mg/L), category) phenotype[a]	Activity at concentration (mg/L)		
		4	8	16
Streptococcus pneumoniae				
11-43B	Penicillin (0.12, I)	Bactericidal[b]	Bactericidal	Bactericidal
30-21B	Penicillin (4, R)	Bactericidal	Bactericidal	Bactericidal
42-4512B	Penicillin (4, R)	Bactericidal	Bactericidal	Bactericidal
Viridans group streptococci				
14-2152A	Penicillin ($\leq$0.015, S)	Bactericidal	Bactericidal	Bactericidal
31-7254A	Penicillin (0.25, I)	Bactericidal	Bactericidal	Bactericidal
63-962C	Penicillin (16, R)	Bactericidal	Bactericidal	Bactericidal
Staphylococcus aureus				
ATCC 29213	Oxacillin (0.25, S)	Bactericidal	Bactericidal	Bactericidal
82-11A	Oxacillin (0.5, S)	Bactericidal	Bactericidal	Bactericidal
82-8A	Oxacillin (>8, R)	Bactericidal	Bactericidal	Bactericidal
Mu50[c]	Oxacillin (>8, R)	Bactericidal	Bactericidal	Bactericidal
CoNS[d]				
15-320A	Oxacillin (0.12, S)	Bactericidal	Bactericidal	Static[e]
82-9A	Oxacillin (0.12, S)	Bactericidal	Bactericidal	Bactericidal
48-388A	Oxacillin (>8, R)	Bactericidal	Bactericidal	Bactericidal
63-294A	Oxacillin (>8, R)	Bactericidal	Bactericidal	Bactericidal
E. faecalis				
ATCC 29212	Ampicillin (2, S)	Bactericidal	Bactericidal	Bactericidal
E. faecium				
69-2323A	Ampicillin (2, S)[f]	Bactericidal	Bactericidal	Bactericidal
15-2666A	Ampicillin (4, S)[f]	Static	Static	Static
15-4011A	Ampicillin (>16, R)	[g]	[g]	[g]
30-7648A	Ampicillin (>16, R)	[g]	[g]	[g]

[a]S, susceptible; I, intermediate; R, resistant.
[b]Bactericidal activity defined by $\geq$3 $\log_{10}$ decrease in the initial inoculum (approximately 5×10^5 to 1×10^6 CFU/mL).
[c]Vancomycin-intermediate *Staphylococcus aureus* MRSA from Hiramatsu et al. [8].
[d]CoNS, coagulase-negative staphylococci.
[e]Bactericidal activity was noted at 8 h, but regrowth occurred to 10^5 CFU/mL at 24 h.
[f]Strain was quinupristin–dalfopristin resistant.
[g]No inhibition was observed, as the BAL9141 MIC parallels the MIC of ampicillin.

described in the *ASM Manual of Clinical Microbiology* [6]. Each experiment was accompanied by a growth control with no antimicrobial agent. A 100-μL sample was used to determine colony counts at 0, 2, 6 and 24 h (streptococci) or 0, 2, 4, 8 and 24 h (enterococci and staphylococci) of incubation. Cidal activity was measured at three concentrations (4, 8 and 16 mg/L) against *Streptococcus pneumoniae* (three strains), viridans group streptococci (three strains), *Staphylococcus aureus* (four strains), coagulase-negative staphylococci (CoNS; four strains), *Enterococcus faecalis* (one strain), and *E. faecium* (four strains), giving 19 strains in total. These concentrations were selected to simulate expected serum peaks for BAL9141 in human subjects that approach 16 mg/L.

Synergistic interactions between BAL9141 and gentamicin were determined against staphylococci (10 strains) and enterococci (two *E. faecalis* and eight *E. faecium* strains) with BAL9141 MIC values of 1 mg/L and without high-level resistance to gentamicin; see Table 2 for susceptibilities to other agents. All staphylococci were resistant to methicillin (oxacillin), and enterococci showed varying degrees of resistance to vancomycin or ampicillin (Table 2). No streptococci were studied because of the favorable cidal activity of BAL9141 when tested alone against these species. BAL9141 was tested at 8 mg/L (8 $\times$ MIC) and gentamicin at MIC/4. Synergy was defined as $\geq$1 $\log_{10}$ (at 4–8 h) and $\geq$2 $\log_{10}$ (at 24 h) CFU/mL greater killing for the combination (BAL9141 + gentamicin) when compared to BAL9141 alone [6].

RESULTS AND DISCUSSION

Streptococcus pneumoniae and viridans group streptococci are generally very susceptible to BAL9141

Table 2 Results of BAL9141 drug interaction (synergy) experiments using combinations with gentamicin (MIC/4) and the time–kill curve method against various resistant and susceptible phenotype strains of staphylococci and enterococci[a]

Organism/strain number	Susceptibility phenotype (MIC (mg/L), category)	Strain origin	Activity for[b]		Interaction category[c]
			BAL9141 alone	BAL9141 + gentamicin	
Staphylococcus aureus					
4-37C	Oxacillin (>8, R)	USA	Bactericidal	Bactericidal	Indifferent
4-111C	Oxacillin (>8, R)	USA	Bactericidal	Bactericidal	Indifferent
55-94A	Oxacillin (>8, R)	USA	Bactericidal	Bactericidal	Indifferent
CoNS[d]					
11-741A	Oxacillin (>8, R)	USA	Bactericidal	Bactericidal	Indifferent
23-1143A	Oxacillin (8, R)	USA	Bactericidal	Bactericidal	Indifferent
31-692A	Oxacillin (4, R)	Canada	Bactericidal	Bactericidal	Synergy
33-779A	Oxacillin (8, R)	Canada	Bactericidal	Bactericidal	Indifferent
48-933A	Oxacillin (>8, R)	Brazil	Bactericidal	Bactericidal	Indifferent
68-1852A	Oxacillin (>8, R)	Turkey	Bactericidal	Bactericidal	Indifferent
78-7893A	Oxacillin (>8, R)	France	Bactericidal	Bactericidal	Synergy
E. faecalis					
15-4006A	Vancomycin (1, S)[e]	USA	Bactericidal	Bactericidal	Indifferent
33-2134A	Vancomycin (1, S)[e]	Canada	Bactericidal	Bactericidal	Indifferent
E. faecium					
25-1368I	Vancomycin (0.5, S)[e]	USA	Bactericidal	Bactericidal	Synergy
52-7395A	Vancomycin (4, S)[e]	USA	Static	Bactericidal	Synergy
55-7032A	Vancomycin (8, I)[e]	USA	Static	Bactericidal	Synergy
33-3259A	Vancomycin (2, S)[e]	Canada	Bactericidal	Bactericidal	Synergy
31-3919A	Vancomycin (0.5, S)[e]	Canada	Bactericidal	Bactericidal	Indifferent
61-8000A	Vancomycin (4, S)[e]	France	Static	Bactericidal	Synergy
66-221A	Vancomycin (0.5, S)[e]	Spain	Static	Bactericidal	Indifferent
95-14745A	Vancomycin (0.5, S)[e]	Germany	Bactericidal	Bactericidal	Indifferent

[a]All strains were tested at a BAL9141 concentration of 8 mg/L. Gentamicin was tested at MIC/4 alone and with BAL9141.
[b]Bactericidal activity was defined as noted in Table 1, footnote b.
[c]Interactive categories were defined as follows: synergy if the combination exhibits $\geq$2 $\log_{10}$ superior killing compared to BAL9141 alone; antagonism if the combination exhibits $\geq$2 $\log_{10}$ inferior killing compared to BAL9141 alone; 'indifferent' was used for all variations between the above-defined extremes.
[d]CoNS, coagulase-negative staphylococci.
[e]These strains were also susceptible to ampicillin (MIC $\leq$8 mg/L).

and exhibit a slightly negative influence of penicillin resistance on BAL9141 MIC results [3]. However, as shown in Table 1, BAL9141 has cidal action against all the streptococci, regardless of their penicillin MIC. Figure 1 exhibits modest concentration-dependent killing by BAL9141, found for a minority of strains at 24 h. There was no difference in the rate of killing at 2 and 6 h of incubation based on drug concentration.

BAL9141 shows cidal action against the *S. aureus* isolates. Oxacillin-susceptible and -resistant isolates, including the vancomycin-intermediate strain Mu50, were killed by BAL9141 [8] with a 4 mg/L concentration sufficient to produce a 3 $\log_{10}$ CFU/mL reduction in the viable cell count. There were reduced killing effects at 8 and 16 mg/L of BAL9141 for some isolates, as indicated in Figure 2. Such 'Eagle effects' [7] were observed in

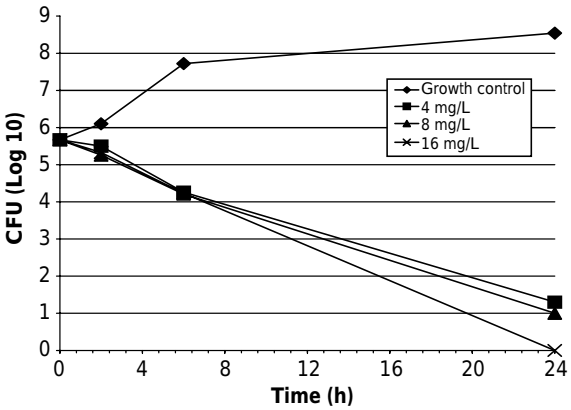

Figure 1 Time–kill curve for *Streptococcus pneumoniae* 42-4512C (BAL9141 MIC, 0.016 mg/L) using BAL9141 concentrations of 4, 8 and 16 mg/L.

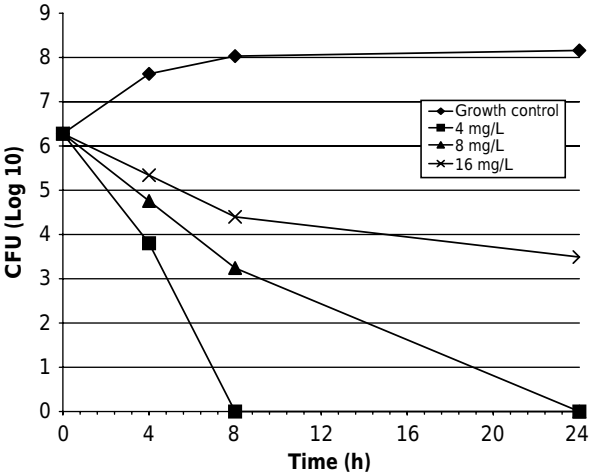

Figure 2 Time–kill curve for *Staphylococcus aureus* 82-11A (BAL9141 MIC, 0.25 mg/L) using BAL9141 concentrations of 4, 8 and 16 mg/L. All concentrations achieved bactericidal action, but an 'Eagle effect' was detected.

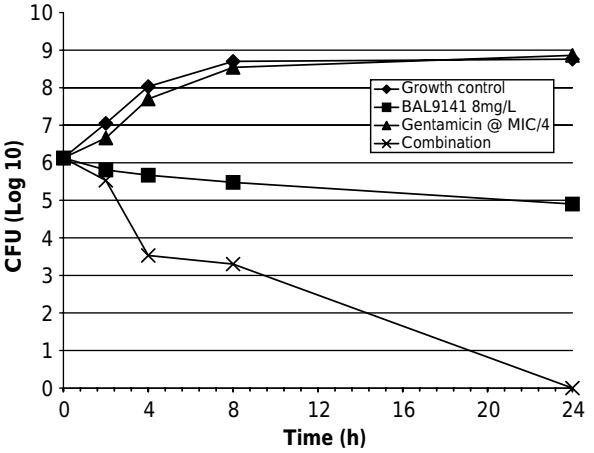

Figure 3 BAL9141 synergy experiment with gentamicin (MIC/4) using *E. faecium* 52-7395A (ampicillin and vancomycin susceptible).

the staphylococci ATCC 29213, CoNS 15-320A and SA 4-11A. One staphylococcal isolate (15-320A) showing cidal action at 8 h with 16 mg BAL9141/L exhibited regrowth at 24 h; this was categorized as a static effect (Table 1).

Bactericidal activity of BAL9141 was observed against ampicillin-susceptible enterococci, while it remained static versus the isolate with an elevated, yet susceptible, ampicillin MIC (15-2666A; Table 1). Ampicillin resistance in *E. faecium* correlates with BAL9141 resistance, so no inhibition was noted in the ampicillin-resistant isolates.

Table 2 shows the assessment of synergistic action between BAL9141 and gentamicin. The combination was bactericidal against all the staphylococci and enterococci tested. For some of the enterococci, it expanded the static action of BAL9141 alone to a bactericidal level. Thus, based on the ≥ 2 $\log_{10}$ CFU/mL superior killing criteria (versus BAL9141 alone) for synergy, the activity of the combination was categorized as either indifferent or synergistic (Table 2). Figure 3 presents a classic example of synergy as observed with the addition of subinhibitory concentrations of gentamicin (MIC/4) to BAL9141 among the enterococci.

BAL9141 showed generally bactericidal action against the streptococci, most staphylococci and ampicillin-susceptible enterococci tested. In our previously reported experience, we found that BAL9141 has only modest activity against enterococci, especially *E. faecium* [3], since it shares coresistance to ampicillin. Some *Staphylococcus* strains exhibited an 'Eagle effect', common to numerous β-lactams. Gentamicin interaction with BAL9141 was categorized as either indifference or synergy. None of the isolates tested showed an antagonistic interaction. BAL9141 alone and in combination showed significant bactericidal activity against resistant Gram-positive pathogens, and these results warrant further clinical evaluation of this broad-spectrum compound. A safe β-lactam active against resistant Gram-positive cocci such as MRSA would be a welcome addition to contemporary chemotherapy in the hospital setting.

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